ON THE USE OF DIETHYLENE GLYCOL IN THE PREPARATION OF BALSAM MOUNTS OF THE MALE GENITALIA OF CERTAIN COLEOPTERA.

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During the past two years, in the course of a revisional study of the North American species of the genus *Helophorus* (Coleoptera, Hydrophilidae), the present writer has had occasion to make a great many mounts and dissections of the male genitalia of the various species of this genus. Early in the study, it became evident that transparent balsam mounts of these structures would be very desirable, but certain complications in dehydrating and clearing the dissections were encountered which, we believe, were primarily due to the fact that we had previously used a 5% solution of ammonia for relaxing and cleaning the specimens.

The male genitalia of *Helophorus* are quite fragile and very thinly chitinized, and it is believed that the ammonia removed some of the natural fats and oils so that serious shrinkage and distortion occurred when the usual methods of dehydration were employed. It was the effort to overcome this difficulty that led ultimately to the use of diethylene glycol as a dehydrating agent, and the choice of this chemical proved to be a happy one. Not only is diethylene glycol an efficient dehydrating agent, but it also clears the tissues so that the dissections may be transferred directly into balsam without further manipulation.

Some explanation of the use of ammonia is perhaps in order at this point. It is a well-known fact that the various species of *Helophorus* secrete a peculiarly stubborn surface exudation in which particles of dirt, mud, and other foreign substances become embedded, with the result that the more minute surface characters are usually almost completely obscured. Dr. David Sharp considers this condition at some length in his "Studies in *Helophorini*" (Ent. Mo. Mag., LI, 1915, pp. 116–117) and states: "Specimens are best cleaned by soaking in very hot water, then washing them with soap and afterwards with benzine." We have never tried Dr. Sharp's method, but for some years have used an aqueous solution of ammonia of a strength of about 5% for this purpose. Ordinary commercial household ammonia averages about 5%.

The use of ammonia for the cleaning of certain Coleoptera was first brought to my attention by my good friend C. A. Frost of

Framingham, Mass., who, in turn, believes that the method originated with Roland Hayward. At any rate, whatever its origin, the present writer has found that ammonia of this strength yields excellent results, and is particularly valuable in cases where a stubborn surface coating is involved. It has been my custom, therefore, to soak specimens of *Helophorus* in a 5% solution of ammonia for a period of fifteen to twenty minutes, after which the surface coating may be rather easily removed, either by means of a camel's hair brush, or with the point of an insect pin. The ammonia softens and swells the coating so that it frequently comes away in large flakes leaving the true surface of the specimens bright and clean. No injurious effects, either to colors or otherwise, have been observed as a result of the use of ammonia in the manner just described. At the same time, prolonged soaking in ammonia will frequently cause pale colors to become permanently darkened. On one occasion, several specimens that were normally of an ochraceous color, were forgotten and remained in ammonia over night. The next morning they had become very dark piceous and subsequent efforts to restore the original color were useless. Another possible objection to the use of ammonia has already been cited, namely, the tendency of the male genitalia to shrinkage and distortion when dehydrated in alcohol series, or by some of the other methods commonly used.

In the case of dried specimens, soaking in ammonia for twenty minutes usually results in a nearly complete relaxation of the specimen, accompanied by a certain amount of softening and swelling of the connective tissues. This is a decided advantage in the genus *Helophorus* since the primary separation of two main divisions of the genus depends upon an accurate count of the number of antennal segments. The counting of these segments often proves to be rather troublesome, since the antennae are usually closely appressed to the under side of the head, and the individual joints are often difficult to see. When ammonia-treated specimens are examined in a drop of the solution under a binocular microscope, the counting of antennal segments becomes relatively simple. It will be found that the antennae may be readily drawn away from the head, and the swelling effect of the ammonia causes the chitinized portion of each segment to become definitely separated from those adjacent to it. Likewise, it is a relatively simple matter to remove the male genitalia of ammonia-relaxed specimens. Because of these obvious advantages, we have been reluctant to discard the use of ammonia in favor of some other cleaning and relaxing agent and; for the same reason, we have sought to overcome the lesser disadvantages involved in its use.

The male genitalia in *Helophorus* are of the usual trilobed type, flattened, so that all of the characters of taxonomic value lie in one plane,[1] and quite small, the average length for all of the North American species known at present being about 0.6 mm. The abdomen in this genus has five visible ventral segments, but closer examination will reveal that there is a sixth segment which is normally concealed beneath the fifth. The male genitalia are located between the tergite and sternite of the sixth segment and, in fresh or in perfectly relaxed specimens, are rather easily extracted with a fine needle or the point of a slender insect pin. Because of the minute size of the structures, the extraction and subsequent handling of the genitalia has been performed under a binocular microscope. The members of this genus show a remarkable lack of secondary sexual characters, and the sexes, except for the average smaller size of the males, can only be recognized with certainty by actual extrusion of the genitalia.

In the preparation of male genitalia for mounting in balsam, the following method seems to work equally well for all specimens regardless of the media in which they may have been collected. We have used this method for specimens that have been collected in alcohol, in ethyl acetate, and for many other specimens, from various sources, for which the collecting medium was wholly unknown. It works equally well for either dried or freshly collected specimens, whether they have been cleaned and relaxed in ammonia or not. In short, we believe that it will prove satisfactory regardless of preliminary technique, so that any one may use it and still adhere to his favorite methods of collection and preparation. Inasmuch as our own method involves the use of ammonia as described above, that is the method that will be described herewith.

The student should provide himself with a dozen pairs of watch glasses, each pair numbered consecutively; also a dozen glass microscope slides numbered to correspond with the paired watch glasses. After the specimens have been cleaned and relaxed in ammonia, a single specimen is removed from the solution, and placed ventral side up under a binocular microscope. A drop of ammonia solution is immediately added so that the specimen is completely immersed. The number of antennal joints is first counted and recorded on a temporary penciled pin-label. Next, the genitalia are extruded, and if the specimen proves to be a female, it is placed in alcohol in one of the watch glasses and covered with another glass which contains

[1] It is obvious that balsam mounts can only be used for genera in which the genitalia lie in the same plane.

the penciled label, locality labels, and other data that may pertain to that particular specimen. Should the specimen prove to be a male, the same procedure is followed with regard to the antennae, next the genitalia are extracted and allowed to remain in ammonia under the microscope, while the specimen itself is placed in alcohol exactly as in the case of the female.

Returning now to the genitalia, the ventral flap and enveloping tissues are teased away with a pair of fine needles, and the dissection is then transferred to a drop of ammonia placed on one of the glass microscope slides. This slide bears the same number as the watch glass in which the male specimen was placed. With the container of diethylene glycol ready at hand, draw off as much of the ammonia as possible with a strip of lintless blotting paper that has been cut to a diagonal point at the tip. As soon as most of the ammonia is drawn off, add a drop of diethylene glycol immediately, and with the point of a needle make certain that the dissection is completely immersed and free of air bubbles. Slide and dissection may now be set aside for dehydration and clearing. It is important to keep the dissection immersed in ammonia right up to the moment that the glycol is applied.

Additional specimens may be carried along in the same way until all of the numbered watch glasses contain specimens. Each male, of course, is kept in its own numbered glass so that it may be re-associated with the genitalia on the slide of corresponding number. Females bearing the same collecting data may be placed together in the same watch glass. Each student can devise his own methods for keeping these important items straight. By the time a dozen specimens have been completed, No. 1 dissection should be ready for mounting in balsam.

We have found that cellulose acetate sheeting[2] is the most satisfactory material for mounting the dissections. For the mount itself, we use acetate sheeting 0.015 inch thick cut into strips 3/16 inch wide. The cover slips are made from 0.010-inch sheeting cut into 1/8-inch squares. The dissection is transferred from glycol directly to the base slip and the excess of glycol drained off by means of a pointed strip of blotting paper. Balsam is added immediately and the cover slip placed in position. Meanwhile, the specimens have been removed from the alcohol and mounted upon paper points in the usual manner. The acetate mounts are trimmed to suitable size and pinned directly beneath the specimen from which each dissection was made. The advantages of this method lie in the fact

[2] Cellulose acetate sheeting should now be obtainable in various thicknesses from the Eastman Kodak Company, Rochester, N. Y.

that the mount takes up no more room than the average locality label and the genitalia are available for examination and study at all times.

The use of diethylene glycol, as a clearing and dehydrating agent in the preparation of insect tissues for mounting in balsam, appears to be new. The advantages attending its use would seem to be obvious. Clearing and dehydrating is accomplished in a single operation and the dissections may be transferred directly to balsam without further treatment. No subsequent deterioration of the mount has been observed when a small quantity of glycol is carried into the balsam with the dissection. Mounts that were made in this manner nearly two years ago are still in first-class condition. As a check, several mounts were made in which the excess of glycol was not drained from the dissection. These mounts, at the end of a year, show a slight iridescence in the balsam which, at the present time, does not greatly impair the transparency of the mount.

Diethylene glycol ($CH_2OH \cdot CH_2 \cdot O \cdot CH_2 \cdot CH_2OH$)[3] is nearly colorless and odorless, with a specific gravity of 1.1318 at 0 degrees centigrade. It is very hygroscopic and will absorb more than its own weight of water at ordinary room temperatures.

Pygmy Grasshopper Notes.—Recently Dr. A. B. Gurney identified a number of pygmy locusts which included the following records:

Tettix acadicus (Sc.). Vernal, Utah, June 17, 1940 (B. A. Haws).

T. subulatus (L.). In Utah at Bear River City, May 5, 1939; Cedar City, June 10, 1938; Soldier Summit, May 16, 1939; Vernal, April 27, 1939; and Woodruff, June 11, 1939, by Knowlton and F. C. Harmston. Other collections included Lewiston (K. and D. E. Hardy), Logan (D. E. Hardy), Logan Canyon (R. E. Nye), Mantua (K. and D. L. Bischoff), and Kanab (K. and W. E. Peay).

Paratettix cucullatus extensus Morse. Riverdale, July 10, 1937, and Monticello, Sept. 5, 1937 (Knowlton); Moab and Roosevelt in June (K. and Harmston); Ogden, July 6, (W. D. Fronk); Logan, August 5, 1903, all in Utah.—G. F. Knowlton, Utah State Agricultural College, Logan.

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[3] I am indebted to the Carbide and Carbon Chemicals Corporation for information regarding the chemical and physical properties of diethylene glycol.